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14. ABSTRACT

Progress has been made on identifying biosensors that will be used to report on the fermentation yields of industrially relevant biological compounds. Screening of the desired chemicals has been completed. Microbes that can utilize these compounds as their sole carbon source have been isolated and archived. Genomic DNA from 97 isolates has been prepared for sequencing. Sixty-nine samples are currently in the sequencing queue but were delayed by about a month because the manufacturer had backordered library construction kits. These materials have now arrived and sequence should be available within one month.

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J. Craig Venter INSTITUTE

Rapid parallel screening for strain optimization

Report Title:	Quarterly R&D Status Report
Report Number:	HR0011-12-C-0062.3
Reporting Period:	November 17, 2012 to February 19, 2013
Contract No.:	HR0011-12-C-0062
Performing Organization:	J. Craig Venter Institute 9704 medical Center Drive Rockville, MD 20850 USA
Principal Investigator:	Chuck Merryman

Abstract

Progress has been made on identifying biosensors that will be used to report on the fermentation yields of industrially relevant biological compounds. Screening of the desired chemicals has been completed. Microbes that can utilize these compounds as their sole carbon source have been isolated and archived. Genomic DNA from 97 isolates has been prepared for sequencing. Sixty-nine samples are currently in the sequencing queue but were delayed by about a month because the manufacturer had backordered library construction kits. These materials have now arrived and sequence should be available within one month.

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Summary

In total, 108 compounds have been used and 85 compounds produced colonies when used as the sole source of carbon and energy. In this reporting period, we have completed screening for microbial growth and nearly finished the proposed sequencing. Sequencing would have been completed except for a delay in required materials from the manufacturer (Illumina). The material has now arrived and sequencing will be completed over the next month.

Introduction

The overall goal in this contract is to link cell-based production to cell survival and thereby make the engineering of new microbial strains that produce industrially relevant biochemicals routine. Recent synthetic biology techniques can make billions of variant cells. Although, many potentially informative mutants are easily made, product yield can only be determined in a few of these. The majority of industrially relevant biomolecules are not chromophores, naturally discernible, or otherwise easily detected. Nevertheless, genetic circuits are capable of linking chemical production to discernible signals such as growth or color intensity. Such a system would allow numerous mutants and mutant combinations to be examined quickly. Genetic circuits needed to screen mutant populations in parallel rely upon the availability of an appropriate biosensor that activates a reporter gene in a product dependent fashion. In this project, genes for two-component and one-component signaling systems (that respond to industrially relevant biomolecules) are identified using microbial growth assays, sequencing, and quantitative PCR (qPCR). To demonstrate that such sensors can be used to maximize product yield, one sensing system will be further engineered. We will reformat this sensor so that it drives expression of a reporter such as an antibiotic resistance marker. This sensor/resistance cassette, and a biosynthetic pathway capable of producing the molecule to which the sensor responds, will be placed within a heterologous host that does not have an overlapping pathway. Basal synthesis of the targeted chemical (by the orthogonal biosynthetic pathway) activates the sensor and increases transcription of the resistance marker (i.e. reporter). In other words, the fermentation product is also the sensor ligand and thus, biosynthesis drives production of the reporter and cell survival. Antibiotic levels in the media will be adjusted so that basal product yield, and hence basal marker activity, is insufficient for survival. Targeted, genome-wide and barcoded alterations to the host genome will then be installed. Variants with better and better chemical production survive by virtue of their ability to withstand increasing antibiotic challenge.

Methods, Assumptions and Procedures

Phylotyping was performed by amplifying 16S rRNA with appropriate primers, cloning the products and Sanger sequencing. Genomic DNA was submitted for sequencing in the middle of December. Sequencing on the Illumina platform was delayed because library construction kits were backordered. The kits have now arrived and sequencing is underway.

Results and Discussion

One-hundred-eight compounds have been used as the sole carbon and energy source (M9 salts supplemented with a target chemical). Colonies were produced for eighty-five compounds (~80%). In addition to M9 salts supplemented with a target chemical, each of the isolated microbes can utilize a standard carbon source such as glucose, glycerol, or succinate. This is necessary for evaluating differential expression and the identification of transcription factors that likely bind to an alternative carbon source. Sixty-nine clones are currently being sequenced. Another 28 clones have been prepared and are ready to be submitted to our sequencing facility. Some carbon sources have more than one clone.

Conclusions

The results continue to indicate that the majority of target chemicals made by organisms are likely to be used as food by other microbes. Bacteria typically utilize the most efficient carbon source available (glucose often being the preferred substrate). More exotic carbon sources are generally subject to catabolite repression and systems for their utilization are activated after preferred carbon sources are exhausted. Besides catabolite repression, sensors are often employed so that the appropriate degradation pathway for a non-preferred carbon source is activated. Nevertheless, how often sensor systems are used cannot currently be estimated because negative results rarely appear in the literature. We have nearly finished generating an extensive data set that will allow us to provide such an estimate. The next step is to evaluate the sequencing results and use functional genomics methods to unequivocally identify sensors and how often they are employed for growth on non-standard carbon sources. This will set the stage for downstream work on the overproduction of such molecules. Identification and experimental validation of specific sensors is indispensable but current results are promising: 1) most target chemicals (~80%) readily produce microbial growth, 2) colony morphology, etc., suggests that different target chemicals resulted in the isolation of different microbial species, 3) automatable procedures can be used if high-throughput screens are needed in the future, and, 4) about 70% of recovered organisms are from the genus *Pseudomonas*, consistent with the commonplace identification of sensors and degradation pathways within this genus when anthropogenic chemicals are used as targets.

Statement of Work Task List:

- Task 1 (Phase I, Year 1, Months 0-3): Completed (please refer to report HR0011-12-C-2.1)
- Task 2 (Phase I, Year 1, Months 4-9): Almost complete. Sequencing of ~96 isolates is underway and will be completed within one month. The report summarizing the results will be generated and submitted following the completion of the sequencing.

Planned Activities for the Next Reporting Period

During the next reporting period we will perform bioinformatics analysis of sequenced genomes, identify transcription factors, and perform qPCR analysis of differential expression. We will rank sensor candidates for further development based on factors such as prior cloning of the pathway in *E. coli*, clarity of the sensor operation from bioinformatics analysis and simplicity of the sensor architecture.

Program Financial Status

	Planned Expend	Actual Expend (Cumulative to Date)	% Budget Completion	At Completion	Latest Revised Estimate	Remarks
Task 1	$59,251	$59,251	100.0%	$59,251	$59,251	Completed
Task 2	$183,957	$69,229	38%	N/A	$183,957	In progress
Task 3	$124,706	$0.00	0.0%	N/A	$124,706	N/A
Cumulative	$367,914	$128,480	35%	N/A	$367,914	N/A

*Expenses for the month of July are not yet available due to the timing of this report.

There is no management reserve or unallocated resources.

Based on the currently authorized work:

- Is current funding sufficient for the current fiscal year? Yes
- What is the next fiscal year funding requirement at current anticipated levels? The budgeted amount for Year 2 of the project is $396,905.25.
- Have you included in the report narrative any explanation of the above data and are they cross-referenced? Not applicable; current funding is sufficient for the current fiscal year.